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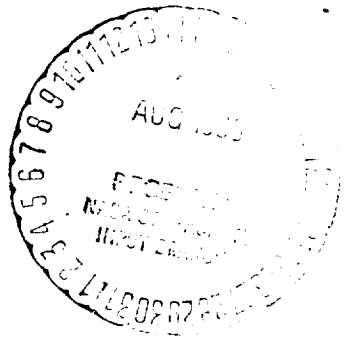

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FURTHER INVESTIGATIONS ON THE SEXUALITY PROBLEM*

R. Hertwig

ABSTRACT. Sex-determining factors affecting the sexual development of the eggs of Rana esculenta, Rana temporaria, and frogs are experimentally investigated. The question of whether the sperm of Rana esculenta affects the sexual development of the egg is the chief subject of this report. Results of the studies indicate that the sperm indeed does exercise a significant influence on sexual determination.

Gentlemen: /55†

Today is the third occasion on which I have sought your attention on a problem which has concerned me very much over the last few years and presumably will continue to do so over the next few years. It can be seen from the complete failures of earlier investigation in part done several decades ago that the question of which factors determine sex in man and animals is so difficult and complicated, that it can be solved only by many years of intensive study. The work of a single individual is insufficient. When I report on the individual stages of my investigation at our meetings, this is done in the hope that I am successful in gaining co-workers, that my communications will stimulate other investigators to direct their attention anew to the sex problem, which has suffered recently from a kind of despair in connection with the work of Strasburger, Lenhossek, Cuenot, O. Schultze, and others. This despair toward further research is last but not least increased by the assertion assumed by most investigators, although completely unproven, that sex is exclusively determined by the egg and is already decided in the ovary of the mother. For if this assertion were correct, then an experimental determination of the sex-determining factors would be an extremely difficult task, i.e. to influence the nutritional relationships in the ovary in a controllable fashion.

When I discussed the question based on general cellular observations, at our Breslau meeting, concerning which influences might play a role in sex determination, I planned a very comprehensive investigational program; I had also tried /56 to deal with the problem in its entire extent from the very beginning. The many technical difficulties with which an exact investigation had to deal, and the very limited facilities available to me forced me, however, very soon to break the task up into a large number of questions so that I might focus on one after the other over a period of years. And so, at our meeting in Marburg, I had only treated the

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question of the influence which postmaturity has on the sex of an egg. Among other things, I showed on an especially illustrative example, a female of Rana esculenta, that the sexual ratio of the eggs at the time of a normal oviposition was 68 (i.e. 68 ♂ to 100 ♀), while with postmaturity of the eggs of three days duration, only males were obtained.

Now, I had been able to ascertain in my earlier experiments that males are exclusively obtained likewise if a premature ripening of eggs is obtained by means of an appropriate experiment, i. e. if the eggs can be caused to leave the ovary earlier than normal and enter the uterus. I concluded from that that the egg changes its sexual character in the course of its development, being masculine in its earlier circumstance, tending toward female at the optimum of its development, and finally becoming masculine again with postmaturity. If from the nest of one frog, both males and females arise, this could be explained by the fact that the eggs at that time are at different stages of development in that they are able to leave the ovary and enter the uterus because of the embrace of the female by the male. Normal fertilization will occur at a time in which the ovary is at a middle stage of sexual tendencies or perhaps deviating only slightly toward the side of the male or female direction (normal maturity of the female). Large deviations toward the male side are obtained if early maturity is introduced or postmaturity is awaited. These considerations allow the possibility of constructing sexual curves, as long as we successfully o b t a i n from one and the same female egg portions at different stages of maturity and fertilize them with the same sperm. Obtaining premature as well as normally mature eggs from one and the same female is not possible as long as we do not find the means of separating the maturity of both ovaries. Therefore, we are forced to study that portion of the sex curve that falls just before the normal maturity and the section following it. I dealt with the second part of the problem in the previous year and studied the changes of eggs after normal maturity. I would like to report on the results obtained from this today. /57

The procedures were the following. Many numbers of pairs of Rana esculenta were placed in roomy separated glass enclosures. As soon as spawning began with one pair, I waited until a sufficient quantity of eggs were laid, and then separated male and female, stored them in a dry and cool environment and allowed them to pair again after 24 hours. The second pairing was also interrupted, as a larger quantity of eggs was laid. In this way three and sometimes even four fertilizations were obtained from the same female.

These cultures were carried out under my direction by my private assistant, Dr. Hans Prandtl, and by him alone, during my absence in the summer vacation period. It was no small task for him to carry out these cultures in an accurate fashion, which included about 30 cultures, some of which had to be kept divided into several containers, and to which were added 10 further cultures for investigation of the influence of sperm. Dr. P r a n d t l was n o t fortunate enough to see the end results of the work and receives my public thanks for his faithful assistance. As you know, my friend and co-worker passed away during a sojourn in Naples, from dysentery -- a great loss not only for me, but for zoological science.

The frogs which I obtained for this culture material came from the Dachau moor, from the region around the town of Lochhausen. The unusual warmth which prevailed at the beginning of May was the cause of the fact that pairing began as early as 10 May in the rapidly-warming water of this area and in the middle of May reached its zenith. In other areas (Dorfen, Schleissheim, etc.), the onset of sexual maturity was delayed about three weeks; spawning was so disturbed by the appearance at the beginning of June of raw, cold weather that I was no longer successful in obtaining further usable material in June, although according to my previous experiences, spawning in the Munich area for Rena esculenta goes on from the end of May to the beginning of June.

The material which became available for use, accordingly was derived from the beginning of spawning. Indeed no artificial prematurity was introduced, as had occurred in some of my earlier experiments, although it can be said that an /58
acceleration of the sex maturity occurred as a result of the abnormal temperatures. That is how I explained the phenomenon that the later fertilizations within the individual developmental series behaved differently this year with respect to mortality than my previous experiments led me to expect. In fertilizations of postmature eggs, i.e. in fertilizations which were carried out three or four days after the female, mounted by the male, had laid its eggs in normal fashion, I had always observed in previous experiments that many eggs, often far more than half, either did not develop at all or showed decided inhibition of development. The egg remained at the blastula stage, or, if gastrulation succeeded, it possessed a yolk plug, which formation of the neural groove impossible. Such inhibitions of the structural development did not occur at all this year in the late fertilization; moreover, there was no greater mortality of the larvae than in the previous first, second and third fertilization. I conclude from this that the high degree of postmaturity which was characteristic of the earlier analogous experiments had not been achieved, although I had selected the same interval of three to four days. Therefore, the starting material this time must have been different from that used before and was less mature.

During the time from 12-13 May, I had set out a total of ten developmental series, which I designated 1 through 10 according to the time of first oviposition. Only a portion of these yielded usable results, series 1, 5, 6, 8 and 10; the others yielded partial or total failures and for various reasons. The disturbance of the spawning leads frequently to a refusal of the male. Often individual packets of eggs are not fertilized, sometimes even a complete oviposition, so that I finally preferred to use artificial fertilization in the final fertilization. In my third series, the fourth oviposition was unfertilized, in the fourth, the second and third ovipositions; in the last case, development proceeded splendidly as I used artificial fertilization in the fourth oviposition. The experiment faces the risk that the female, despite the fact that it is separated from the male, and is kept cool and dry, continues to spawn. The second series would be made useless. Finally, there are cases in which the egg material proves to be unusable in the /59
experiment. Series VII and IX had to be abandoned because all fertilizations, even the normal ones, gave unfavorable results. For series IX, perhaps the very unusual size of the eggs was to blame.

In presenting to you my results, I will consider first series I, VI and X, because in these, sex determination of the dead or dying larvae could be performed at an early time in the development with great certainty and because among them, all cultures which were set out developed splendidly; sex could be determined for half of the starting material, some times even for three-quarters. These are the best results which could be obtained. To illuminate this, I mention only one i t e m: Born, using Rana temporaria, which is much easier to cultivate, brought only an eighth of his larvae, to a point, as he believed, where he could determine their sex.

I will give the results of the culture in tabular summary and in double fashion for each series: In an upper row the actually-found numbers, underneath this, the numbers of the sex ratios derived from them, which are expressed as is known in such a way, that the calculation relates to how many males are produced per 100 females. The Roman numerals designate the fertilizations, the numbers inserted between them, the number of hours which had gone by from one fertilization to the next. In developmental series I and X it was possible to obtain four, and in series VI, only three, fertilizations.

	I.	II.	III.	IV.
	— 6 —	— 18 —	— 30 —	
1.	34 ♀ : 47 ♂	65 ♀ : 77 ♂	156 : 194	7 ♀ : 48 ♂
	141 %	119 %	124 %	685 %
	— 36 —	— 18 —		
6.	64 ♀ : 61 ♂	101 ♀ : 139 ♂	115 ♀ : 169 ♂	
	95 %	137 %	147 %	
	— 18 —	— 24 —	— 22 —	
10.	55 ♀ : 52 ♂	148 ♀ : 87 ♂	71 ♀ : 70 ♂	17 ♀ : 129 ♂
	100 %	59 %	100 %	759 %

The table above shows in convincing fashion that the sexual character of the eggs changes in the course of time. For no two successive fertilizations show the same sex ratio. In fact, the differences are so large that it is impossible to call them chance phenomena. The change shown in the table in the characteristics of the eggs is completed in the uterus and has nothing to do with the nutritional conditions in the ovary. For it is known that the release of the eggs from the ovary and their transition into the uterus takes place in a very short time; the process completes itself so quickly that it is not at all easy to discover the eggs during their transfer into the uterus in the body cavity. In any case, the eggs from a female which has begun to spawn have been transferred into the uterus for a long time. Thus, the prevalent opinion that the sex is already determined in the ovarian egg once and for all is definitively refuted. /60

That these are not chance results also derives from the fact that, from a certain period of time, in which the optimum for the female sex is given, the sex ratio is displaced in regular fashion, and indeed in favor of the male sex. The

high degree of postmaturity of the eggs which I achieved in my earlier experiments was never obtained in any earlier experiments. I conclude from this that among those only as little as half the eggs were capable of development, while all others had died as a result of inhibitory forces. In the latter the mortality was not different in any way from the first, second, and third fertilizations. The fact that the number of examples investigated in the four fertilizations of the first series was so small, resulted therefore from the situation that the relevant female frog contained only a little over a hundred eggs in the uterus, when it was killed for the purposes of artificial fertilization on the third day of the investigation. Several of these eggs were obviously mechanically damaged and died very early.

The difference in comparison to earlier experiments is explained from the circumstance that the earlier damaging postmaturity was avoided this time and that the final fertilization did not yield exclusively males. Nevertheless, here too, especially in series I and X, the percentage increase of males was extremely large (685% in the first, 759% in the second series).

The three series under discussion differ furthermore from each other in that the optimum for the female sex was obtained in series 6 even at the first fertilization, while in series 1 and 10, it appeared only at the second fertilization. This not only does not contradict my opinion but even is a necessary postulate of the same. As has already been derived from the very fact that the spawning period of frogs stretches from eight days to three weeks depending on /61
the weather in the region, the frogs in one and the same area can be found in various stages of maturity. Thus it will occur that completely mature males will pair with females which are still behind in maturity, therefore causing a somewhat early transferral of eggs from the ovary into the uterus and causing a d e g r e e of maturity such as I had obtained in earlier experiments, in great measure. Prematurity favors, however, as has been mentioned in earlier experiments, the appearance of males. This explanation was brought home in the present case by two factors especially. First, the spawning period of Rana esculenta was advanced by at least 14 days in comparison to the usual situation as a result of unusually warm weather in the previous year. Besides this, the first fertilization of the first series showed -- and this was shown by an especially high percentage of males -- much similarity with early fertilization as an extraordinary number of eggs died off, which was not the case with the second fertilization which took place on the same day. An extraordinary degree of early maturity for natural circumstances certainly explains the striking preponderance of males in all of the fertilizations of the first series.

On the basis of my very incomplete experiences, I suggested in my earlier lecture that it was probable that an ascending and descending curve could be expected for the percent ratio of males, if it were possible to obtain fertilized eggs from one and the same female at different degrees of early maturity, furthermore at normal maturity and at different degrees of postmaturity. Even though it was not possible to obtain a complete curve of this kind by means of my observed material, nevertheless, the numbers which I obtained in series I and X correspond to my expectations insofar as they yield at least a small part of the descending l e g of the curve and the entire ascending part. I had suggested furthermore that two fertilizations separated in time could under

certain conditions lead to very false conceptions of the sexuality with respect to the character of the sexuality curve which I would expect. It would be conceivable that two fertilizations by chance would correspond exactly to two corresponding points on the increasing and decreasing part of the curve. This possibility is most elegantly illuminated by fertilization series X and IV. If I had had only the first and third fertilizations from fertilization series X which represent a time interval of 42 hours, then I would have obtained a sex ratio of 100 in both cases. In fertilization series 3 the hypothetical case constructed here occurred in fact. Of the four fertilizations of this series, the second was lost through an unfortunate accident (overturning of the container), the fourth was not obtained, because the eggs laid under the influence of the male remained unfertilized and did not develop. So only fertilizations 1 and 3 were investigated, and these two gave the sex ratio of 100. /62

	I.		II.		III.		IV.
	— 20 —		— 28 —		— 20 —		
3.	92 ♀ : 92 ♂				68 : 68		
	100 %				100 %		

A further characteristic of the cultures which were raised from postmature eggs that I would like to mention is that they, insofar as they developed at all normally, revealed a greater degree of growth energy than normal cultures. This struck me especially in the first fertilization series and led me to undertake weighing of the polliwogs at the time when the hind legs began to develop. On the same day, 94 polliwogs of the first, 146 of the second, and 57 of the fourth fertilization were weighed; they gave the following weights: I. 68.5 g; II. 101.7 g; IV. 89.5 g. The polliwogs of the first and second fertilization therefore weighed on the average 0.7 g, those of the fourth, 1.6 g. The first were for the most part without extremities or rarely had only moderately strong hind legs; the polliwogs in the fourth fertilization, except for two which had only stumps of feet, had in contrast well-developed strong hind legs. In general, metamorphosis occurred earlier in the polliwogs raised from postmature eggs than in the other material.

Under some circumstances the size of the young frogs at the time of metamorphosis appears to be dependent on the degree of maturity of the eggs. In the culture which I discussed in Marburg (1 9 0 6 culture), the young frogs of the normal culture were significantly smaller than those from the very postmature eggs. However, this example is not completely flawless. In fact, I am not sure whether the males used in the fertilization of the postmature eggs were the same as the males of the first fertilization. My experiments, however, have shown me that eggs which are fertilized with various kinds of sperm can have a variable growth energy. /63

While a sex ratio change could be shown in the three series discussed in the individual fertilizations with certainty, three other series yielded uncertain results. The relevant three series differ from the first three by a very striking indifference in the morphology of the sex organs. While the sex of R. esculenta can usually be determined with certainty in young polliwogs,

in which the hind extremities are still undifferentiated plaques, it was not possible for me, in this case, to differentiate males from females in fully-metamorphosed animals, not even in animals which had been fed far beyond metamorphosis. The sex glands had a unique morphology deviating from the usual. As I have discussed earlier, the testis or ovary is laid down under the usual circumstances as a fine thread running along the kidney. When this develops into a testis, then the entire caudal portion regresses, the foremost portion, bordering on the fatty bodies thickens to a flat oval body. In the female sex, in contrast, the entire length of the genital ridge develops to a richly-folded glandular body. In the cultures under consideration, the sex glands in all cases showed the same morphology after checking 100 individuals, which was similar in characteristic to neither ovaries nor testes. They were strikingly small; every one was divided into two sides by a right-angle bend, the anterior, which extended to the border of the fatty body, and the caudal one, which lay near the kidneys. Histologically, the organ was reminiscent of the sex glands as they appear in a large portion of freshly-metamorphosed young grass frogs, which Pfluger took for hermaphroditism and which he further assumedly found developing later into males. One of my students, Marcell-Schmitt, confirmed this point of view of Pfluger for Rana temporaria by detailed investigation. The general prevalence of this situation in many development series of Rana esculenta and the circumstance that all individuals of a nest behaved similarly makes it appear that the possibility is ruled out that the described characteristic sex glands in R. esculenta would likewise yield only males. I believe moreover that they represent a situation of indifference, from which development can proceed as well toward the female as toward the male animal.

Of the three fertilization series, among which the described indifferent /64
situation of the sex organs occurred, one series developed extraordinarily well; I was successful in obtaining four fertilizations, all of which turned out well, of which the fourth occurred 52 hours later than the first. Surprisingly, I found in all animals obtained from the four fertilizations the totally identical characteristic of the sex apparatus; by means of postmaturity there was no displacement toward the masculine side. And still I believe this would have come out the s a m e if I h a d raised these animals from the stage of indifference and beyond to the time of definitive sexual differentiation. I conclude this from the third fertilization series in which the indifference of the sexual apparatus was likewise apparent, but not as distinctly. In this third indifferent culture, fertilizations 2 and 3 were unsuccessful, fertilizations 1 and 4 which were separated by 55 hours had developed well. The main group of individuals were also of indifferent nature here, although there were still individuals which exhibited m o r e or l e s s definite characteristic sex glands, either female or male. In the male individuals, the uppermost side of the sex gland was differentiated into a testis, the lower side was rudimentary, or the portion lying in the corner had taken on the form and size of a testis, while both ends were rudimentary. In the female individuals, the lower side was more definitively developed, the upper, rudimentary. A sharp distinction of the different conditions of form was not possible, because they were connected by all possible transition forms. Nevertheless, the material can be classified somewhat into males (♂), indifferent forms, for which I used the double sign ♂♀, and more or less clear females (♀). The first fertilization

yielded 83 ♂, 90 ♀♂ (intermediary forms), 40 ♀, the fourth fertilization 90 ♂, 61 ♀♂, and 10 doubtful ♀. In this case as well, a gradual displacement of the sexuality character developed in the fourth fertilization, in that the ratio of males to females plus indifferent forms had increased from 64 to 127, while the females apparent in the first fertilization had as much as disappeared. Nevertheless, this displacement of the sexual ratio in comparison to those earlier-discussed, larger changes, does not mean much. It appears that the changes in the sexual apparatus in indifferent forms proceeds much more slowly than in animals with definitive sexual tendencies; the direction in which the changes are completed, however, is equivalent. Postmaturity favors here also the formation of the male sex. /65

The striking phenomenon that the tendencies toward male development in postmature eggs proportional to the degree of postmaturity increase, were explained in my lecture at the Marburg meeting a year ago, from the phenomenon of artificial parthenogenesis. Just as any eggs which are treated with strychnine, nicotine, magnesium chloride, fatty acids, etc., obtain the ability to develop without fertilization, a certain degree of parthenogenetic development capability is induced in eggs by means of long-standing absence from fertilization as well. I assume that the frog eggs had achieved this degree through postmaturity. Parthenogenesis favors the development of the male sex, as can be seen in hymenoptera. In some cases in which fertilized eggs yield females only, parthenogenesis in these cases yields only males (vesparia and apiaria); in other cases, fertilization yields only females, parthenogenesis, males and females (gallicole Hymenoptera). In a third kind of cases, finally, not only fertilization yields females, but parthenogenesis as well; in such cases, many generations are required before the cumulative effect of parthenogenesis is enough to produce males.

This chain of ideas which was offered in Marburg has in the meantime found new support in the elegant investigations of Herbst. He is treating sea urchin eggs with materials that bring about parthenogenesis, and fertilizing them as well, before parthenogenetic development had begun. In this way, he obtained a situation in fact, which I assume has appeared in my experiments on frog eggs as well: Eggs in which a certain tendency toward parthenogenesis had already appeared, which however could still be fertilized. It turned out then, that the parthenogenetically-stimulated eggs, although they were fertilized later, and the blastomeres contained half paternal and half maternal chromatin, developed only maternal characteristics. First the parthenogenetic development introduced had a very significant negative effect on the later fertilization. Common to both experiments is the reduced effect of the sperm. The consequences of this have been studied by both of us from different points of view, by Herbst from the standpoint of the inheritance of paternal and maternal characteristics, by me from the standpoint of the problem of sex determination. If we combine the results of both series of experiments, then we gain an insight into the processes of crossed inheritance, that male offspring in general show a tendency to be similar to the mother. Reduction of the paternal inheritance indicates an approach to parthenogenesis, and must therefore favor, like the latter, formation of the male sex, simultaneously, however, favoring the inheritance of maternal characteristics. /66

This chain of thoughts which I am developing here is only understandable with the prerequisite that the sperm has a certain share in the sex determining. Personally, I believe that experiences with propagation of hymenoptera prove this, that in fact spermatozoa can exercise an influence on sex determination, from which, however, it would not follow that they exercise it in general. As I discussed above, I find myself opposed to the prevalent opinion with these interpretations, which deny any influence of the male sex cells on sex formation. I have always wondered on what uncertain basis such an assertion is based which bears on such an important problem. It is known that there are primarily two phenomena which are used for proof, although both have no meaning at all in evaluating our problem. The first phenomenon has been known for a long time: that twins which are derived from one and the same egg have the same sex. Since these twins arise from the separation of the first two blastomeres of one and the same egg, which was fertilized by the same spermatozoon and developed under completely equivalent conditions, then this case illuminates only the trivial truth that likes subjected to the same conditions produce likes. The second phenomenon for which Dinophilus apatris is usually given as the characteristic example, which, however, occurs in other animals, is given by the fact that it can be seen even before fertilization which eggs will develop into males and which into females. The female eggs are differentiated from the rudimentary, small male egg by their enormous size. That such a case of extreme differentiation has no bearing on our problem is clear. A /67
sex-determining influence which might be derived from the spermatozoa could of course be suppressed by an extreme increase in the influences derived from the egg. In this regard, I have already referred to the relationships of Dinophilus.

The question whether sperm have any sex-determining influence or, to use the vernacular, whether the father participates in the sex of the children, is very old. Many extensive discussions have been written about it without any results being obtained, corresponding to the enormous effort invested in solving the question. The reason for lack of success is to be found in the applied investigational methods. The attempt was made to decide this question in mammals and in fact by means of statistical methods, instead of experimentally with a choice of appropriate subjects. Such subjects are represented by animals which permit artificial fertilization, and which are not difficult to raise and in which the sex can be determined. Among our local fauna, the forms which correspond to these preconditions are only amphibians and fish.

I have chosen Rana esculenta; the eggs of a female were divided into five portions with care being taken in each case that every portion represents the content of the left as well as the right uterus and also equivalent amounts from both ovaries; these five portions were fertilized with sperm from five different males. In order to control the series derived from these five fertilizations by means of a parallel culture of the same egg material, I used the eggs of a second female and fertilized them exactly the same way with the sperm of the five males used in the first series. I wanted to use this experiment which demanded much effort in completion simultaneously to answer a second question. I had available frog pairs from different regions, from the region of Lochhausen where the spawning time was approaching its end, and from the region of Schleissheim, where running had just begun. The frogs of both localities showed

different habitus, especially different sizes. I wanted to determine whether the minor local differences which were apparent would be expressed in fertilization also. Therefore, I chose one Lochhausen and one Schleissheim female and three Lochhausen and three Schleissheim males. If we designate the former as L and S and the latter as l^1, l^2, l^3 and s^1, s^2, s^3, then there are twelve fertilization possibilities of which two (Ll^3 and Ss^3) were not used, as the following table shows. /68

Ll^1, Ll^2, Ls^1, Ls^2, Ls^3
Sl^1, Sl^2, Ss^1, S^2, Sl^3

Eight of these fertilizations form two parallel series insofar as two kinds of eggs are fertilized in each case by the same sperm, while for the ninth and tenth fertilizations (Ls^3 and Sl^3) the above-stated condition did not apply in that the eggs as well as the spermatozoa were chosen differently. This latter occurred in order, if I may say so, to increase the number of cross-fertilizations; this turned out later to be useless since the parallel series offered a very special interest.

As long ago as our Marburg meeting, I was able to report that the developmental behavior of the egg was influenced in a high measure not only by their own constitution but from the various characteristics of the spermatozoon. Indeed, none of the ten cultures developed so favorably as the best esculenta cultures used to develop. Nevertheless, several showed a satisfactory course, while others in contrast yielded from the very beginning despite very careful attention very poor results. At that time, I had already suggested that the Schleissheim eggs developed more poorly in general than the Lochhausen eggs.

Even more striking was the influence of certain males. The data reported a year ago that all eggs fertilized with males s^1 and l^1 and in fact not only those from Schleissheim but those from Lochhausen as well had developed especially poorly, was continued this year. Culture Ss^1 even died out completely, and indeed very early. The animals in this culture did not orient themselves vertically with their adhesive disks as is otherwise customary at very early developmental stages among frog larvae, moreover, they did not take any nourishment, so that the culture container was always free of fecal material, while well-breeding cultures always demonstrate a thick floor layer of feces. Likewise culture Ls^3 died out very early.

A third assumption which I expressed in Marburg that cross-fertilization might exercise an unfavorable influence on development did not bear out. The differences which were apparent in percentage ratios of mortality between "bastard cultures" and normal cultures were not insignificant, however balanced out in the course of the cultures, so that finally even a "bastard culture," Ls^2, yielded the largest number, while a normal culture (Ss^1) died out completely. In any case, the individual differences of eggs and spermatozoa completely overwhelm the differences caused by different origin, inasmuch as the latter plays any role at all. /69

The large differences in development which eggs of the same mother show with respect to the sperm used made it apparent to me from the beginning that the individual cultures would differentiate themselves significantly in sex ratio. This impression turned out to be correct in a fashion exceeding all expectations; it turned out the spermatozoa exercised a very energetic influence on the development and sexual differentiation of the sex glands.

In such a situation it is naturally difficult to decide what in the development of the sex glands of the daughter animals is to be attributed to the characteristic of the egg and what to the spermatozoa. Nevertheless, I believe from the patterns yielded by the various fertilizations that at least something can be read from it, something which it is correct to view as a consequence of a certain characteristic of the egg, because it recurred in all fertilizations. After this, the eggs of the Schleissheim and Lochhausen females would have had a similar characteristic, a characteristic which I observed often and would like to designate as a situation of sexual indifference. I found it in three of the above-described ten fertilization series in which I set out to study the influence of postmaturity, and described already more precisely in what way it is expressed in the sex apparatus of the young animal. I will repeat in short the most important part. Only the upper part of the genital ridge develops; this consists of two sides which meet at a right angle or run into each other in a gradual curve; one side lies, as is customary for sex glands, upon the kidneys, the other, in contrast, lies along the border of the fatty body which lies post-caudally. I found this indifferent situation in every one of the eight cultures; it was present in very characteristic form sometimes only in a few, sometimes in many individuals; in other individuals it was modified and indeed according to the sperm used in different ways, in some cultures toward the female, while in other cultures toward the male side. As above, I will make known in the following the indifferent condition of the sex apparatus by juxtaposing the sign of the male and the female.

The results of the sex determination are expressed in the following table: /70

	l^1	s^1	l^2	s^2
L.	2 ♀♂ : 31 ♀	9 ♀♂ : 53 ♀	80 ♂ : 77 ♀♂	176 ♂ : 156 ♀♂
S.	9 ♀♂ : 65 ♀	†	77 ♂ : 73 ♀♂	10 ♂ : 75 ♀♂

	l^3	s^3
L.	—	†
	9 ♂ 59 ♂♀ : 65 ♀♂ 11 ♀	—

In this table I have expressed the fact that two cultures died out by the symbols † and - : Ss^1 and Ls^3, that two possible further cultures were not set out from the start, Ll^3 and Ss^3, so that in toto eight cultures were investigated. Of these, the four cultures Ll^2, Sl^2, Ls^2, Ss^2 showed a somewhat favorable developmental course which was already apparent from the numbers derived from the individuals studied in the sex determination, while Ll^1, Sl^1 and Ls^1 demonstrated a n enormous mortality. The cultures which developed well showed simultaneously a definitive male character, in that practically no

females were apparent, while, however, very many males appeared, more males than indifferent forms. Only culture Ss^2 contained few males and consisted almost exclusively of indifferent forms. The rest of the cultures which were difficult to raise but survived the investigation contained almost exclusively females, and at the same time very few indifferent forms, and in contrast practically no males. Culture $S1^3$ had an intermediary character with 9 males, 59 quasi-male indifferent forms, 65 quasi-female indifferent forms, and 11 females.

I believe it can be seen as a certain result of the investigation that in the case before us the different sperm in fact exercise a very significant influence on the sexual differentiation. For, if we were to say that differences in the cultures were caused by the large mortality especially in the case of the last-named culture, then the very paradoxic assumption would have to be made that despite complete equivalency of the cultural conditions in one part of the culture, all females, and in another part, all males died out. The attempt to refer the variability of the cultures to chance is ruled out, if the significant numbers of animals used is considered; moreover, it is further ruled outby the very striking phenomenon that the different distribution of sexes was the same essentially in the L cultures and in the S cultures. /71

It is a somewhat different question whether it is justifiable to draw general conclusions from the two described experimental series. The great influence which the different kinds of sperm exercise in the cases before us is perhaps to be explained by the fact that the sexual character of the eggs was in such a labile equilibrium that even a weak influence emanating from the sperm was enough to tip the balance toward one or the other side. It would be conceivable that as a rule the eggs at the time of fertilization are sexually determined to such a high degree that the relatively small influence of the sperm is hardly relevant. This can be said with certainty only after extensive experiments. Therefore, this year I have been occupied with the solution of this task exclusively and will report the results of the extended experimental series, set up for this purpose, after conclusion of the cultures.

For another reason, I have decided to repeat the fertilizations of frog eggs with sperm which derive from various males. I believe that in this way it will be possible to form a clearer conception of the nature of the factors which are decisive for whether a fertilized egg develops into male, a female, or a hermaphrodite. Let us assume first, which is of course by no means proven, that the fertilized egg is determined sexually, then this is only to say that the later sex of the animal which comes out of the egg is present in anlage form. How should we conceive of this anlage? In the literature as far as I know, this question is not even asked, let alone any attempt having been made to answer it. Nevertheless, I believe that the prevalent conception leads in that direction, that sex-determining substances for male and female are assumed to be in the egg. Thus, according to whether one or the other is preponderant the individual derived from an egg will be either male or female. If both sex cells, egg and sperm, would contain the sex-forming substances, then the sex would be determined by the addition of both.

As I have discussed in the introductory paragraphs of my lecture at Breslau, I do not conceive of the sex-determining factor so simply, but rather as a consequence of very complicated regulatory processes of cell life, in /72
which the affinity and the mass ratio of nuclear substance to cellular substance play an important role. I would like to not only develop my interpretation once again, but refer to further ideas.

Parallel fertilizations of eggs of several females with the spermatozoa which derive from various males will be appropriate to shed light on the question asked. Let us assume that we have fertilized the eggs of females A, B and C with spermatozoa of males 1, 2, 3, 4 and 5; then, organized by the eggs, we would have three fertilization series:

A 1.	A 2.	A 3.	A 4.	A 5.
B 1.	B 2.	B 3.	B 4.	B 5.
C 1.	C 2.	C 3.	C 4.	C 5.

For each of these series, the five numbers expressing the sex ratio would be different from each other and could be plotted on a curve, assuming that the sperm, just as in the experiment which I performed last year, would exercise a modifying influence on sex. If sex were to be determined by the fact that the sex-determining substances of the spermatozoa are added to that of the eggs to obtain a common result, then the curve would have to give three parallel lines derived from the three series given above, whose distance would be determined exclusively by the difference in the eggs. If the curves did not correspond to these conditions, then the assumption of additive sex materials of male and female or similar factors would be contradicted. Care would have to be taken in this attempt to exclude from judgment all cases in which the sex-determining power of the eggs was so great that the sperm could not exercise any modifying influence even under the most favorable conditions.

If we tested the results gained from last year's investigations from the point of view discussed above, then we would find that this material was inappropriate because of the small numbers of sex determination due to the unfavorable course of the cultures. By and large, there is a certain parallelism of both fertilization series. Still, the result of the fertilizations with sperm s^2 does not quite fit, insofar as the eggs of Lochhausen females yielded many males, while those of the Schweissheim females only very few.

And I would like to raise one further point of view for new questions: /73
In investigations on the influence of postmaturity of the sexual product on sex, I have always spoken only of postmaturity of the eggs. Since, however, the fertilizations always made use of the same males, it would be necessary to speak of a postmaturity of the sperm and the possibility would arise that postmaturity of sperm might have an effect on altered sexuality. However, since according to all previous experiences the sperm are not as alterable as the eggs and have a larger resistance, I consider it not very likely that much significance could be ascribed to the postmaturity of the sperm in altering the sex ratio. Nevertheless, this problem could be solved only by means of exact

experiments. This demands a very complicated organization, but lies in the realm of the possible.

Discussion

Prof. Kobert (Rostock):

A criticism which might be leveled against the experiments with eggs derived from four different ovipositions separated temporally and following one another might be that the female eggs develop to maturity more slowly and lose their ability to develop more quickly. For this reason, the first oviposition yields only a few females and the last oviposition likewise only a few females.

Prof. Hertwig:

Against the criticism of Prof. Kobert, I would like to remark that the differences in maturity among the eggs in the case before us can partly be applied. The transition of the eggs from the ovary into the oviduct and the beginning of egg maturity which begins at that time is stimulated by the embrace on the part of the male. The process occurs simultaneously for all eggs and occurs so quickly that it is difficult to find even a few eggs in transition from ovary to oviduct to uterus.

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